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ANTI-OXIDANT AND ANTI-BACTERIAL ACTIVITIES OF SINAPIS ALBA L. (LEAVES, FLOWERS AND FRUITS) GROWN IN SYRIA

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This research aims to determine the phenolic and flavonoid contents in the extracts of the Sinapis alba L. parts and to calculate the free radical scavenging and antibacterial activity of them. The highest phenolic content was 28.302 mg/g dry plant, the highest flavonoid content was 2.072 mg/g dry plant in the methanolic flower extract. In interaction with diphenyl picryl hydrazyl, the inhibitory concentrations of half of free radicals ranged from 2.836 mg/mL in the methanolic fruit extract to 17.853 mg/mL in the chloroform flower extract. Methanolic extracts showed a zone of inhibition against Staphylococcus aureus, the diameters of the inhibition zone were 17.2 mm in leaf and fruit extracts, and 15 mm in flower extract at a concentration of 700 mg/mL. The study showed that the methanolic extracts of Sinapis alba L. have good phenolic and flavonoid contents, good free radical scavenging activity and have antibacterial activity against Staphylococcus aureus.

INTRODUCTION

Plants have been used in the treatment of many diseases and they have formed an important resource for medicinal preparations[1]. The family Brassicaceae (Cruciferae) contains over 330 genera and about 3,700 species including mustard which importance has increased recently. There are three species of mustard: White mustard (*Sinapis alba* L.), brown mustard (*Sinapis nigra* L.) and oriental (*Sinapis* juncea L.)[2].

Sinapis alba L. is a widespread plant in the Mediterranean region and has great economic and medical importance due to its anti-tumor and insecticidal properties[3]. The major compounds of *Sinapis alba* L. are glucosinolates, fatty oil, proteins and phenyl propane derivatives. It has been used in the treatment of common cold, bronchitis, rheumatism and in the treatment of inflammation of the respiratory tract and the gastrointestinal tract in homeopathy, but it should be avoided in children under 6 years, gastrointestinal ulcers and inflammatory kidney diseases[4]. Mustard has high amounts of antioxidants, vitamins (B complex) and minerals (Calcium, magnesium, iron, potassium and selenium)[5].

Sinapis alba L. seeds contain of fatty acid (41.3% Erucic acid), glucosinolates, and phenolic acids (*P*-Hydroxy benzoic acid, *trans*-Sinapic, *trans*- Caffeic, and *trans*- Ferulic)[6]. Kaempferol is the main flavonoid in the leaves extract of the plant, and there are isorhamntin and quercetin[7]. Phenols and flavonoids have antioxidant activity, these natural antioxidants have anti-cancer properties, inhibit apoptosis and reactive oxygen species (ROS) generation and have free radical scavenging activity[8]. Mustard leaves extract decreases the damage of oxidative stress by reducing the level of oxygen radicals[9].

White mustard has anti proliferative, proapoptotic, antioxidant and antimicrobial properties. It can be used as a food preservative because mustard glucosinolates reduce bacterial growth in salad packages, which leads to increasing vegetables' shelf life. In addition, it can be regarded as a part in anticancer therapy

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because of its low toxicity[10]. The plant has antibacterial activity against *Staphylococcus aureus* and no activity against *Escherichia coli* and *Pseudomonas aeruginosa* because Gram-negative bacteria are more resistant than Gram-positive bacteria[11].

The aim of this study was to compare between leaves, flowers and fruits of *Sinapis alba* L. which is widespread in Syria and estimate the phenolic and flavonoid contents in the extracts of parts of the plant as well as to determine the 2,2-diphenyl-1picryl hydrazyl (DPPH) scavenging activity and antibacterial activity against some selected pathogenic bacteria.

MATERIALS AND METHODS

Plant material

The plant was collected between January and April 2019 between 8 and 9 AM from different areas of Damascus and its countryside. It was identified by Dr. Imad Kadi, Department of Plant Biology, Faculty of Science, Damascus University.

The plant parts (leaves, flowers, and fruits) were separated from each other and dried at room temperature for 14 days in the shade and were powdered in an electric grinder (Multimaxy, GR-1000, Taiwan) to prepare the plant extracts.

Preparation of plant extracts

Plant parts powder (25 g) was placed in the Soxhlet device, and the extraction was performed by adding 600 ml of solvent (methanol 70% and chloroform), at 60º C, for 6 hours, the extract was filtered with filter paper (Zelpa, Belgium). Solvent was removed using a rotatory evaporator under vacuum at 60º C (RV 10 digital IKA, Germany) and then put in the shaking incubator (JSSI-100C –JSR, India) at 40º C with stirring until the weight was relatively constant. The crude extracts were stored at 6º C in airtight containers. The process was repeated three times for each sample and yields were calculated. The extraction yield was calculated by the following equation:

Yield%= (weight of dry extract/ weight of dry powdered plant material) × 100.

Determination of total phenolic contents in the plant extracts

The method published by Abdeltaif S *et al.* was applied to determine total phenolic contents in samples[12]. 20 µl of extract was mixed with 1.58 ml of distilled water and 300 µl of 20% sodium carbonate and 100 µl of Folin-Ciocalteu reagent (Merck KGaA Darmstadt, Germany). The blank was concomitantly prepared, containing 2 ml ethanol. The samples were mixed and then left in a dark place at room temperature for 45 min, the absorbance was measured at 765 nm (UV-VIS Spectrophotometer (T80+), PG Instruments, United Kingdom). The total phenols were identified by a calibration curve of gallic acid in ethanol within the concentration range 0-500 mg/l (Fig.1). Means were calculated from three parallel analyses as gallic acid equivalents in mg/g of dry plant.

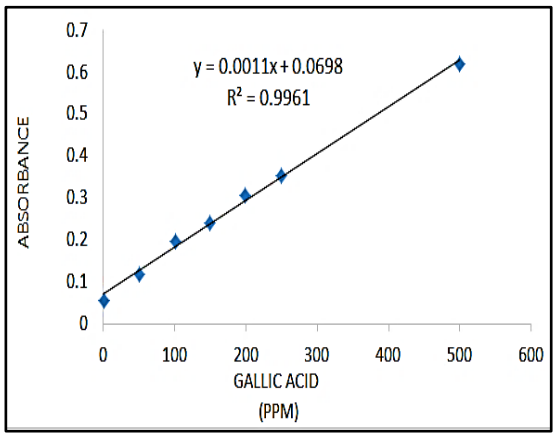

Fig.1: Calibration curve of gallic acid.

Determination of total flavonoid contents in the plant extracts

Aluminum chloride colorimetric method was used to determine total flavonoid contents in samples as mentioned by Kitaz A, 2017[13]. One ml of extract was mixed with 1 ml of aluminum trichloride 2% (Riedel-de Haen, Germany). The blank was concomitantly prepared, containing 2 ml methanol. The samples were mixed and then left in a dark place at room temperature for 30 min, the absorbance was measured at 464 nm (UV-VIS Spectrophotometer (T80+), PG Instruments, United Kingdom). The calibration curve was produced within the concentration range 0-40 mg/l of quercetin (Fig.2). Means were

calculated from three parallel analyses as quercetin equivalents in mg/g of dry plant.

Evaluation of DPPH scavenging activity

The free radical scavenging capacity of *Sinapis alba* L. extracts against DPPH (Tokyo Chemical Industry, Japan) was measured based on Alhajali O *et al.*, 2021 method[14]. 300 µl of each concentration of extract was mixed with 3 ml of 45 µg/ml ethanolic solution containing DPPH radicals. The samples were mixed and then left in the dark for 30 min, and the absorbance was measured at 518 nm (UV-VIS Spectrophotometer (T80+), PG Instruments, United Kingdom). The calibration curve was produced within the concentration range of 9-175 µg/ml of ascorbic acid (Fig.3). Results were calculated by preparing a series of concentrations (0.5- 1- 2- 3- 4- 5 mg/ml) from each extract and showed as IC_{50} (the inhibitory concentration of half of free radicals). The percentage of scavenging activity was calculated using the following equation:

DPPH scavenging activity

$$(\%) = (A_{control} - A_{sample}) / A_{control} \times 100.$$

Where:

$A_{control}$: The absorbance of control (Ethanol + DPPH)

A_{sample}: The absorbance of sample (Extract + DPPH).

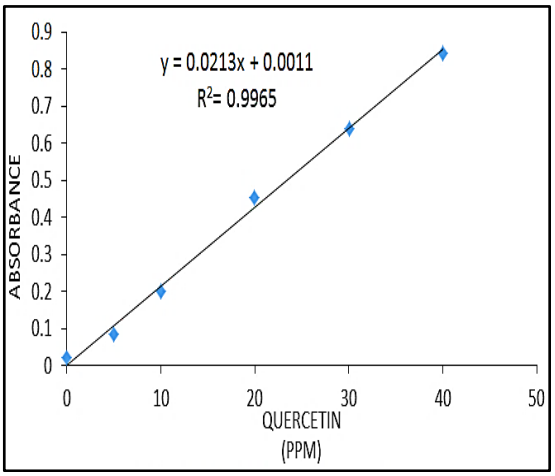

Fig. 2: Calibration curve of quercetin

Bacterial strains

Pathogenic bacterial strains (*Staphylococcus aureus* as Gram - positive, and *Escherichia coli, Pseudomonas aeruginosa* as Gram-negative) were isolated and identified in the Microbiology Laboratory, Department of Plant Biology, Faculty of Science, Damascus University. Strains were used to estimate the antibacterial activities of methanolic extracts of *Sinapis alba* L.

Antibacterial activity

The method reported by Perez C *et al.* was applied to examine *Sinapis alba* L. methanolic extracts against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* by agar well diffusion method[15]. The nutrients broth agar (Liofilchem, Italy), was inoculated with the selected bacteria and incubated for 24h at 37º C. Sterile cotton swabs were dipped in the bacterial suspension (the microbial turbidity was equal to 0.5 McFarland) and uniformly streaked over the entire surface of the Mueller-Hinton agar. This procedure was repeated by streaking three times and rotating the petri dish about 60 degrees after each time. Finally, the rim of the agar was swabbed. Dry methanolic extracts were dissolved in absolute Dimethyl Sulfoxide (DMSO) to prepare a series of concentrations (200, 300, 500, 700 mg/ml). Four wells with a diameter of 7 mm were punched in each plate with a sterile cork borer. 60 µl of extract was introduced into the well[16]. Antibiotic susceptibility testing discs (Cefaclor, levofloxacin, tobramycin, and gentamicin) were used as positive controls, while DMSO was used as negative control. All petri diches were incubated for 24 h at 37º C. The antibacterial activity of the *Sinapis alba* L. methanolic extracts was interpreted as inhibition zone diameters surrounding the wells (mm).

Statistical analysis

SPSS software program, version 26 was used for statistical analysis.

Two-Way Analysis of Variance (ANOVA) test was used to define the significance of differences between *Sinapis alba* L. parts in the determination of total phenols and flavonoids contents and free radical scavenging capacity.

T-Test was used to define the significance of differences between *Sinapis alba* L. parts and ascorbic acid in the determination of free radical scavenging capacity.

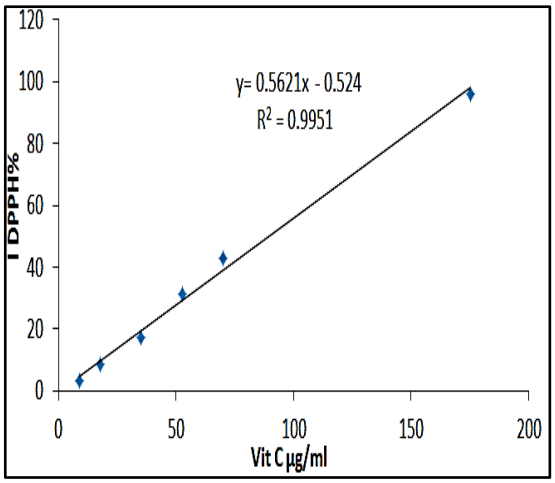

Fig. 3: Calibration curve of ascorbic acid.

One-Way ANOVA test was used to define the significance of differences between *Sinapis alba* L. parts in antibacterial activity.

For all previous tests, significance levels were $P< 0.05$.

All tests were repeated three times and displayed as mean value ±standard deviation using Microsoft Excel 2013.

The Pearson's correlation was performed for the relationship between extract concentration, total phenolic content, total flavonoid content and DPPH scavenging activity in each plant part. Correlation is significant at the 0.01 level.

RESULTS AND DESCUSSION

RESULTS

Yield of extraction

Yield of extraction ranged from 41.31% in the 70% methanolic flower extract to 4.39% in the chloroform extract of the fruits (Table1).

Determination of total phenolic and flavonoid contents

Phenolic compounds ranged from 28.302 mg gallic acid equivalents/ g dry plant in the methanolic flower extract to 1.596 mg gallic acid equivalents/ g dry plant in the chloroform extract of the fruits (Table1).

Flavonoid compounds ranged from 2.072 mg quercetin equivalents/ g dry plant in the methanolic flower extract to 0.446 mg quercetin equivalents/ g dry plant in the chloroform extract of the fruits (Table1).

Anti-Oxidant activity of Sinapis alba L. parts

Inhibitory Concentration (IC50) ranged from 2.836 mg/ml in the methanolic fruit extract to 17.853 mg/ml in the chloroform extract of the flower (When calculating the percentage of inhibition at a concentration 2 mg/ml it was ranging between 16.449 and 35.189%) while ascorbic acid had IC50 value 0.09 mg/ml (Table1).

Pearson's correlation was 1.000 between extract concentration, total phenolic content, total flavonoid content each other's, while it was 0.793 between DPPH scavenging activity and other factors (Table 2).

There were no zones of inhibition against *Escherichia coli*.

Diameter of the inhibition zone against *Staphylococcus aureus* ranged between 12.2 mm in flowers extract to 17.2 mm in leaves and fruits extracts.

Diameter of the inhibition zone against *Pseudomonas aeruginosa* ranged between 7.5 mm at 200 mg/ml concentration to 10 mm at 700 mg/ml (Table3).

Table1: Phenol, flavonoid contents, and IC_{50} value of *Sinapis alba* L. parts and positive control.

Solvent	*Sinapis alba* L. part	Yield %	Phenols (mg gallic acid equivalents/ g dry plant)	Flavonoids (mg quercetin equivalents/ g dry plant)	IC_{50} (mg/ml)
Methanol 70%	Leaves	32.15 ± 0.85	19.282 ± 0.222	2.068 ± 0.008	3.963 ± 0.001
	Flowers	41.31 ± 2.11	28.302 ± 0.467	2.072 ± 0.014	2.872 ± 0.001
	Fruits	33.42 ± 0.10	22.007 ± 0.393	1.354 ± 0.012	2.836 ± 0.001
Chloroform	Leaves	6.52 ± 0.24	2.179 ± 0.023	1.425 ± 0.002	7.374 ± 0.002
	Flowers	7.52 ± 0.39	1.811 ± 0.012	0.458 ± 0.003	17.853 ± 0.002
	Fruits	4.39 ± 0.09	1.596 ± 0.007	0.446 ± 0.003	10.972 ± 0.090
Ascorbic acid					0.09 ± 0.000

Values are mean ±standard deviation

DISCUSSION

Yield of extraction

Yields of methanolic extracts were higher than chloroform extract yields, this may be due to the plant material, which contains elevated levels of polar compounds that dissolve in methanol because of its higher polarity than chloroform[17].

Determination of total phenolic and flavonoid contents

The importance of these calibrations is to determine the differences between Sinapis alba L. grown in Syria and global Sinapis alba L.

Results of total phenolic contents agree with the content of Vergun O et al., 2019 who studied ethanol leaf extract of white mustard that contains total phenols content 73.58 mg gallic acid/ g dry extract[18]. Nevertheless,

Zhang D et al., 2019 estimated total phenols content of ethanol seeds extract to be 53.2 mg gallic acid/ g dry extract, which is lower than the previous results[19].

Total flavonoid contents of this study were lower than flavonoids found by Vergun O et al., 2019 which were measured as 62.91 mg quercetin/ g dry extract, this may be caused by different plant environment or different calibration method[18].

Anti-Oxidant activity of Sinapis alba L. parts

Methanolic fruit extract had the highest free radical scavenging activity, this may be due to the high content of antioxidants in seeds which are in the fruits (like vitamin E, sterols, phosphatides and omega 3)[20].

Free radicals scavenging activity results are compatible with Ronak F, 2016 and Mayengbam S et al., 2014[21&22].

The percentage of inhibition of free radicals in white mustard seed methanol extract was calculated by Mayengbam S et al., 2014, it was 39% at concentration 2 mg/ml[22]. However, the percentage of inhibition of white mustard powder ranged from 15 to 36% at a concentration of 1 mg/ml according to Ronak F, 2016[21].

The statistically significance level value of Two-Way ANOVA Test was (P= 0.000< 0.05), which indicates that there was a statistically significant difference between methanolic and chloroform extracts.

The statistically significance level value of T-Test was (P> 0.05) in methanolic extracts, which indicates that there was not a statistically significant difference between methanolic extracts and ascorbic acid.

Table2: Pearson's correlation between extract concentration, total phenolic content, total flavonoid content and DPPH scavenging activity

	Extract concentration	Total phenolic content	Total flavonoid content	DPPH scavenging activity
Extract concentration	1	1.000**	1.000**	0.793**
Total phenolic content	1.000**	1	1.000**	0.793**
Total flavonoid content	1.000**	1.000**	1	0.793**
DPPH scavenging activity	0.793**	0.793**	0.793**	1

**Correlation is signifiant at the 0.01 level

Table3: The zone of inhibition of *Sinapis alba* L. extracts and controls against selected bacteria

Sinapis alba L. part	Concentration	Diameter of inhibition zone (mm)		
		Escherichia coli	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
Leaves	200 mg/ml	ND	14.0 ±0.0	7.5 ±0.0
	300 mg/ml	ND	15.0 ±0.0	8.0 ±0.0
	500 mg/ml	ND	16.0 ±0.0	9.0 ±0.0
	700 mg/ml	ND	17.2 ±0.2	10.0 ±0.0
Flowers	200 mg/ml	ND	12.2 ±0.2	7.5 ±0.0
	300 mg/ml	ND	13.0 ±0.0	8.0 ±0.0
	500 mg/ml	ND	14.0 ±0.0	9.0 ±0.0
	700 mg/ml	ND	15.0 ±0.0	10.0 ±0.0
Fruits	200 mg/ml	ND	13.2 ±0.2	7.5 ±0.0
	300 mg/ml	ND	15.0 ±0.0	8.0 ±0.0
	500 mg/ml	ND	16.0 ±0.0	9.0 ± 0.0
	700 mg/ml	ND	17.2 ±0.2	10.0 ± 0.0
Cefaclor	30 µg	ND (R)	35.0 ±0.0 (S)	ND (R)
Levofloxacin	5 µg	9.0 ±0.1 (R)	29.0 ±0.2 (S)	31.0 ± 0.0 (S)
Tobramycin	10 µg	11.0 ±0.0 (R)	20.0 ±0.3 (S)	19.0 ± 0.2 (S)
Gentamicin	10 µg	13.0 ±0.2 (I)	21.0 ±0.3 (S)	21.0 ± 0.0 (S)
DMSO	-	ND (R)	ND (R)	ND (R)

Values are mean inhibition zone (mm) ±standard deviation
ND: Not Detected. R: Resistance. I: Intermediate. S: Sensitive

The statistically significance level value of T-Test was ($P< 0.05$) in chloroform extracts, which indicates that there was a statistically significant difference between chloroform extracts and ascorbic acid.

Extract concentration, total phenolic content, total flavonoid content and DPPH scavenging activity in each plant part showed significantly positive correlation. This means that concentration of extract; total phenols and flavonoids have an important role in DPPH scavenging activity of Sinapis alba L. because poly phenols have a reducing role due to its hydroxyl groups, which gives it hydrogen-donating property[23]

Anti-Bacterial activity of Sinapis alba L. parts

The zones of inhibition with diameter less than 12 mm were considered as having no antimicrobial activity, diameters between 12 and 16 mm were considered moderately active and with 16 mm were considered highly active according to Sujatha A et al., 20135. These mean no antimicrobial activity against Escherichia coli and Pseudomonas aeruginosa.

All extracts have moderate activity against Staphylococcus aureus except leaves and fruits extracts, which at 700 mg/ml concentration, have high activity.

Gram-negative bacteria (Like Escherichia coli and Pseudomonas aeruginosa) have an envelope which is made of three layers (Outer membrane, peptidoglycan cell wall and inner membrane). This envelope gives bacteria impermeable and resistant properties24.

These results are similar to Camacho C et al., 2019 and Ronak F, 2016, but Weldu H et al., 2019 displayed higher activity against Staphylococcus aureus and Escherichia coli[11&21&25].

According to previous studies the increasing of extract concentrations have a direct relation to give a high antimicrobial activity due to the increase in phenols, flavonoids and glucosinolates contents[26].

The statistically significance level value of One-Way ANOVA Test was (P= 0.151> 0.05), which indicates that there was not a statistically significant difference between the extracts of plant parts, the reason could be that all parts have similar concentrations of the active compounds which are given the antimicrobial activity.

Conclusion

This study concluded that the methanolic extracts of Sinapis alba L. have good phenolic and flavonoid contents, good free radical scavenging activity and have anti-bacterial activity against Staphylococcus aureus but not against Escherichia coli or Pseudomonas aeruginosa. There was no statistically significant difference between the plant parts studied, so any part of them can be used in the food and pharmaceutical industries.

Acknowledgment

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نشرة العلوم الصيدلية
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النشاط المضاد للتأكسد والمضاد للجراثيم في أوراق وأزهار وثمار نبات الخردل الأبيض النامي في سوريا

رشا خطيب – كنان المكي

قسم العقاقير والنباتات الطبية ، كلية الصيدلة ، جامعة دمشق ، دمشق ، سوريا

يهدف البحث إلى المقارنة بين أجزاء الخردل الأبيض الذي ينمو في سوريا ، وتحديد المحتوى الفينولي والفلافونوئيدي في الخلاصات بالإضافة إلى حساب النشاط الكاسح للجذور الحرة والمضاد للجراثيم. بلغ أعلى محتوى فينولي ٢٨,٣٠٢ ملج مكافئ من حمض الغاليك/ج نبات جاف ، وأعلى محتوى فلافونوئيدي ٢,٠٧٢ ملج مكافئ كيرستين/ج نبات جاف في خلاصة الأزهار الميثانولية، بينما بلغ أقل محتوى فينولي ١,٥٩٦ ملج مكافئ من حمض الغاليك/ج نبات جاف ، وأقل محتوى فلافونوئيدي٠,٤٤٦ ملج مكافئ كيرستين/ج نبات جاف في خلاصة الثمار الكلوروفورمية.

في التفاعل مع مركب دي فينيل بيكريل هيدرازيل تراوحت التركيزات المثبطة للأكسدة بين٢,٨٣٦ ملج/مللي في خلاصة الثمار الميثانولية و١٧,٨٥٣ملج/مللي في خلاصة الأزهار الكلوروفورمية . أبدت الخلاصات الميثانولية هالات تثبيط لجراثيم العنقوديات المذهبة قطرها ١٧,٢ مم في خلاصتي الأوراق والثمار، و١٥ مم في خلاصة الأزهار عند تركيز ٧٠٠ ملج/مللي . أظهرت الدراسة أن خلاصات نبات الخردل الأبيض الميثانولية تمتلك محتوى فينولياً وفلافونوئيدياً جيداً، ونشاطاً كاسحاً للجذور الحرة ، وفعالية مضادة لجراثيم العنقوديات المذهبة.